CONDITIONAL PETITION FOR EXTENSION OF TIME

If entry and consideration of the amendments above requires an extension of time, Applicants respectfully request that this be considered a petition therefor. The Commissioner is authorized to charge any fee(s) due in this connection to Deposit Account No. 14-1263.

ADDITIONAL FEE

Please charge any insufficiency of fees, or credit any excess, to Deposit Account No. 14-1263.

REMARKS

Applicants respectfully request reconsideration and allowance of this application in view of the amendments above and the following comments.

At the outset, Applicants respectfully request that the finality of the Office Action be withdrawn. On page 2 of the Office Action, the claims are rejected for the first time under 35 USC § 112, second paragraph, as being indefinite in their use of the term "tackiness." On page 5 of the Office Action, a statement is made that the new grounds of rejection in the Office Action were necessitated by Applicants' amendment. However, since original claim 2 used the word "tackiness" in the next-to-last line thereof, it is clear that this rejection could have been raised against original claim 2 in the first Office Action, but wasn't. Consequently, the Examiner's

position that Applicants' last amendment necessitated this new rejection is simply untenable. In view of the foregoing, Applicants request that the Examiner withdraw the finality of the open Office Action. In the event that a formal petition is necessary to accomplish this result, then Applicants respectfully request that this be considered a petition. No additional fee is believed to be due in connection with such petition, but, if it is, then the Commissioner is hereby authorized to charge such fee to Deposit Account No. 14-1263.

Applicants also point out that the amendments above cancel claims 8-11, which relate to the use of phospholipids to increase the stability of a cosmetic or dermatological preparation comprising chitosan. Consequently, these amendments on their face materially reduce the issues for possible appeal and, therefore, Applicants do not believe that entry and consideration of these amendments requires any stringent showing under 37 CFR § 1.116(b) as to why the amendments above are necessary and were not presented earlier. In view of the foregoing, Applicants that the Examiner should enter and consider these amendments. An early notice that these amendments have been entered and considered is earnestly solicited.

Claims 4-7 were rejected under 35 USC § 112, second paragraph, as being indefinite. In the middle of page 2 of the Office Action, the Examiner professes a lack of understanding of what is intended by the term "tackiness." In response, however, Applicants note that the Examiner himself provides a definition for the term in the last sentence on page 4 of the Office Action. Consequently, the term has a well known definition and, therefore, its use cannot be

indefinite.

With respect to the Examiner's criticism that the specification does not contain a formal definition for this term, Applicants would remind the Examiner that, as stated by the Court in *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 94 (Fed. Cir. 1986), "a patent need *not* teach, *and preferably omits,* what is well known in the art (emphasis added)." Since the term "tackiness" has a well known definition, as conceded by the Examiner himself, in view of *Hybritech,* the specification need not teach, and preferably omits, such definition.

Finally, with respect to the Examiner's criticism that it is unclear how much tackiness is decreased, Applicants submit that there is no requirement that the specification include such information. Tackiness is a matter of feel and ultimately what is important is that by following the teachings of the present invention users thereof will feel that the inventive compositions are less tacky than are compositions that do not incorporate the inventive combination of chitosans + phospholipids. The extent of such reduction is not important as any reduction in tackiness is beneficial.

In view of the foregoing, Applicants submit that the Examiner would be fully justified to reconsider and withdraw this rejection. An early notice that this rejection has been reconsidered and withdrawn is, therefore, earnestly solicited.

Claims 4-11 were rejected under 35 USC § 102(b) as being anticipated by EP 0 771 556 or Magdassi, U.S. Patent No. 5,518,736, or FR 2 667 072. As indicated above, claims 8-11 have been canceled. Therefore, the remaining claims are claims 4-7, which are drawn to a method of reducing tackiness by incorporating a phospholipid into a cosmetic or dermatological preparation comprising chitosan.

Applicants previously argued that the Examiner had not shown where in these cited references the instant methods were taught. In the middle of page 3 of the Office Action, the Examiner says "[t]he differences argued cannot be determined since applicant has not provided an English translation. The reference appears to teach the stability of the composition by chitosan."

In response, Applicants have a number of comments. First, EP 0 771 566 and Magdassi, U.S. Patent No. 5,518,736, are in English. Accordingly, no translation of these references was needed in order to ascertain the correctness of Applicants' arguments. Applicants submit that neither of these references teaches what is presently claimed, i.e., that tackiness can be reduced by incorporating a phospholipid into a cosmetic or dermatological preparation comprising chitosan. Accordingly, Applicants respectfully request that the Examiner immediately withdraw the rejection of claims 4-7 over these references.

Second, with respect to FR 2 667 072, an English-language translation thereof is attached.

Applicants submit that there is no teaching or suggestion therein that tackiness can be reduced by incorporating a phospholipid into a cosmetic or dermatological preparation comprising chitosan. Accordingly, Applicants respectfully request that the Examiner immediately withdraw the rejection of claims 4-7 over this reference as well.

Third, the Examiner is apparently in agreement is this assessment already since he expressly found that the teachings of these references were limited to stability. The Examiner himself has not found any indication in any of these references that tackiness can be reduced by incorporating a phospholipid into a cosmetic or dermatological preparation comprising chitosan.

In view of the foregoing, Applicants submit that the Examiner would be fully justified to reconsider and withdraw this rejection. An early notice that this rejection has been reconsidered and withdrawn is, therefore, also earnestly solicited.

Claims 4-11 were rejected under 35 USC § 103(a) as being obvious over EP 0 771 556 or Magdassi, each taken individually or in combination. At the bottom of page 4 of the Office Action, the Examiner comments:

> "With regard to tackiness, there is no specific definition of this term in the specification and applicant himself has not shown that the tackiness is reduced. The dictionary meaning of tackiness is 'sticky' and applicant has not shown that the prior art compositions are sticky. [Emphasis added.]"

In response, Applicants submit that this is an attempt to impermissibly shift the burden from the Examiner to Applicants. As the proponent of this rejection, the burden was squarely on the Examiner to make out a *prima facie* case of obviousness; only when the Examiner's burden was carried forward did the burden then shift to Applicants to provide any proof of nonobviousness. *In re Piasecki et al.,* 223 USPQ 785, 788 (Fed. Cir. 1984). Further, absent a showing that Applicants' allegations that the inventive compositions reduce tackiness were incredible or unduly speculative, the Examiner was required to accept these allegations as true. *In re Marzocchi et al.,* 169 USPQ 367, 369 (CCPA 1971). Thus, Applicants were not required to show that tackiness is, in fact, reduced or that the prior art compositions are sticky. Instead, the burden was on the Examiner to show that the prior art taught or suggested that tackiness could be reduced by incorporating a phospholipid into a cosmetic or dermatological preparation comprising chitosan. Since the Examiner has not made such a showing, Applicants submit that the rejection is not substantiated and should be withdrawn. An early notice that this rejection has been reconsidered and withdrawn is, therefore, also earnestly solicited.

Claims 4-11 were rejected under 35 USC § 103(a) as being obvious over FR 2 667 072 alone or in combination with EP 0 771 556. In response, the arguments made above in response to the first obviousness rejection apply with equal force here. There is no teaching or suggestion in FR 2 667 072 alone or in combination with EP 0 771 556 that tackiness could be reduced by incorporating a phospholipid into a cosmetic or dermatological preparation comprising chitosan. Consequently, the combination of FR 2 667 072 and EP 0 771 556 also does not make out a

prima facie case of obviousness, and, therefore, the Examiner should withdraw this rejection as well. An early notice that this rejection has been reconsidered and withdrawn is, therefore, also earnestly solicited.

Applicants believe that the foregoing constitutes a bona fide response to all outstanding objections and rejections.

Applicants also believe that this application is in condition for immediate allowance. However, should any issue(s) of a minor nature remain, the Examiner is respectfully requested to telephone the undersigned at telephone number (914) 332-1700 so that the issue(s) might be promptly resolved.

Early and favorable action is earnestly solicited.

Respectfully submitted,

NORRIS McLAUGHLIN & MARCUS, P.A.

By _____
Kurt G. Briscoe
Reg. No. 33,141

220 East 42nd Street
30th Floor
New York, New York 10017
Phone: (212) 808-0700
Fax: (212) 808-0844

CERTIFICATE OF MAILING

I hereby certify that the foregoing Amendment under 37 CFR § 1.116 and the accompanying English translation of FR 2 667 072 are being deposited with the United States Postal Service as first class mail in an envelope addressed to: Box AF, Commissioner of Patents, Washington, D.C. 20231, on the date indicated below:

Date: February 20, 2001 By _____
Kurt G. Briscoe

TRANSLATION: French Pat. Appln. 2 667 072
Applicant: BIOETICA Société anonyme, France
Inventors: Alain Domard and Sandrine Demarger
Date laid open to the public: 27 March 1992

Title: **Ternary complex of chitosan, of calcium ions and lipids, method of preparation and their applications.**

Abstract:
The invention relates to a stable ternary complex of chitosan, lipid and calcium.
The lipid can be in the form of a monovalent salt of alkaline metal, preferably sodium.
This complex can be used to stabilize aqueous dispersions of liposomes.
This complex can have an infrared spectrum such as the one observed in Figure 2.

Ternary complex of chitosan, calcium ions and lipids, method of preparation, and applications thereof.

The present invention relates essentially to ternary complex compounds of chitosan, calcium ions and lipids, their method of preparation and their applications.

It is known that chitosan is a polysaccharide obtained by the N-deacetylation of chitin, an important constituent of the exoskeletons of all arthropods.

Chitosan has already been used as a biological material offering numerous applications in the fields of medicine, pharmacology or agricultural foods.

In the medical field, these properties of biodegradability, non-toxicity, local hemostasis, non-antigenicity, etc., have provided it with outlets in applications of cicatrization and cell culture as described in R.A.A. Muzzarelli, G. Biagini, A. Pugnaloni, O. Filippini, V. Baldassare, "Reconstruction of parodontal tissue with chitosan," Biomaterials, 1989, 20, 598-603.

These hypocholesterolemiant properties have been demonstrated in the rat, in alimentary application, as described in M. Sugano, T. Fujikawa, V. Hiratsuji, Y. Hasegawa, "Hypocholesterolemic effects of chitosan in cholesterol-fed rats." Nutrition reports international, 1979, 19, 327-34.

Likewise, these growth stimulant and bioprotective properties have been revealed in the agricultural field as described in R.E. Lewis, "Treatment of plants with salts of chitosan," International Patent, WO89/07395, or more generally in plant biology, as described in H. Hauss, W. Jeblock and A. Domard, "The degree of polymerization and N-acetylation of chitosan determine its ability to elicit callus formation in suspension cells and protoplasms of catharanthas roseus." Plants, 1989, 178, 385-92.

It is also known that the lipids and the calcium ions play a part in most biological mechanisms, whether in the animal or vegetable domain.

The present invention is based on the unexpected discovery that it was possible to prepare stable ternary complex compounds of chitosan, lipids and calcium ions, and that these stable ternary complexes exhibited a synergetic effect making it possible to arrive, when it is desired, at a partial or complete mastery of the control of essential biological mechanisms.

The present invention therefore has as its principal object to resolve the new technical problem consisting in providing a solution making it possible to control the calcium ion, lipid or chitosan content of a medium, in an independent or simultaneous manner.

The present invention also has the purpose of solving this new technical problem in a simple, reliable and repeatable manner, thus enabling the proposed invention to be used in a considerable number of applications, particularly the purification of waste water, agriculture, the food farming industry, dietetics, particularly through reduced-fat diets, cosmetology, particularly by means of external thinning preparations by cutaneous application, and medicine, particularly for the treatment of certain lesions.

The present invention also has the purpose of resolving the new technical problem by providing a method permitting the removal from or the addition to a medium of chitosan, a lipid or calcium, in an independent or simultaneous manner.

All these technical problems are solved for the first time by the present invention in a satisfactory, simple, reliable and repeatable manner, being thus usable on an industrial scale.

Thus, under a first aspect, the present invention provides a stable ternary complex compound of chitosan, lipid and calcium.

In one particular embodiment, this ternary complex is characterized in that the lipid is in the form of a monovalent salt of alkaline metal, preferably a sodium salt.

In another variant embodiment, this ternary complex is characterized in that the lipid is formed by a saturated or unsaturated fatty acid, particularly a phospholipid such as lecithin.

In another particular embodiment, this ternary complex is characterized in that it is in the form of a polynuclear complex wherein the ratio of the number of calcium atoms to the number of chitosan or lipid molecules is greater than 1, and particularly between more than 1 and 4.

In another particular embodiment, the ternary complex has a molecular ratio of lipid:calcium ion:chitosan or 1:1:1 or 2:1:1 or 3:1:1.

It can be observed that the ternary complex according to the invention is stable and is formed between the $-NH_2$ functions of the glucosamine patterns of chitosan, the calcium ions and the anionic sites of the lipids, i.e., either the carboxylate functions $-COO^-$ and/or phosphate in the case of type $P-O^-$ phospholipids.

The formula of the complex can be given, without limitation, as follows:

$$[-NH_2Ca_m\,(COO)Nn]^{(2m-n)^+}$$

wherein $-NH_2$ represents an amine function of chitosan and COO represents an acid function of the lipid.

This ternary complex may be defined as being of two types, either the mononuclear type when m = 1 (a single calcium ion), with n varying from 1 to 7, particularly from 1 to 3, and the polynuclear complexes wherein m is greater than 1, particularly is greater than 1 and less than 10, still better, between 4 and 10.

In another particular feature of the ternary complex is that it is characterized in that the chitosan shows a degree of acetylation below 30%, and preferably has a residual acetylation rate below 0.5%, being thus essentially completely deacetylated.

According to another particularly advantageous feature of the invention, the chitosan has a molecular mass running from the molecular mass of the oligomer to polymers having a molecular mass above 5,000.

Furthermore, according to another particular characteristic of the invention, the calcium ions are supplied by any calcium salt that is soluble in water at the pH used. The anion associated with the calcium ion will of course depend on the chosen application and may in particular be a chloride. Any other anion may be used to the extent it does not interfere with the formation of the desired ternary complex.

According to a second aspect, the present invention likewise provides a process for preparing a stable ternary complex of chitosan, lipid and calcium, characterized in that the chitosan, the said lipid in the form of a monovalent salt of alkaline metal, and calcium ions, are brought together. In particular, they are brought together by dissolving or suspending them in an aqueous solution, particularly at a pH between 5.5 and 6.5, approximately.

According to a particular embodiment of the process the combining mentioned above comprises the introduction of the chitosan in the free amine form, particularly in the solid state, into a solution containing calcium ions and the lipid in the form of monovalent salt.

In another particular embodiment of the process, the combining mentioned above comprises first of all the formation of a liposomal solution of the aforementioned lipids, then the addition to this solution of the calcium ion, then the addition of chitosan either in the form of a solid in suspension or in dissolved form in an aqueous solution.

In another particular embodiment of the process, the combining mentioned above comprises first of all the dissolution of the chitosan in an aqueous solution whose pH is between 5.5 and 6.5, approximately, to which the calcium ions are then added, particularly in a concentration close to that of the glucosamine residue of the chitosan, and then lastly a solution of the lipid mentioned above in the form of monovalent salt.

In a particular variant embodiment of the process, the amount of lipid added is increased progressively for the same initial calcium concentration.

In another particular variant embodiment of the process, the initial ratio of calcium ions to the number of NH_2 groups of the chitosan is high, being at least equal to 4, and being preferably between 4 and 10, thus forming polynuclear complexes obtained with at least 4 calcium ions per NH_2 group of the chitosan and 1 mol of lipid.

According to a third aspect, the present invention also furnishes a method for stabilizing an aqueous dispersion of liposome, characterized in that a stable ternary complex is formed by the addition of chitosan and calcium ions to the said liposome suspension so as to form a stable ternary complex of chitosan and calcium with lipids constituting the liposomes.

According to this third aspect, the liposomes can be stabilized for the fields of cosmetology and pharmacy, the liposomes then encapsulating the active principles, of medicine in medical applications wherein the liposome plays an important part alone or by the active principles which contain it, as well as in the field of agriculture where the stabilization of liposome alone or encapsulating an active principle [is] used as a growth stimulant or bioprotector.

Likewise, under a fourth aspect, the present invention concerns the use of the stable ternary complex mentioned above as an individual or simultaneous reserve of chitosan, lipid and calcium, particularly in order to control the flows of calcium or exchange them at the surface of a medium, as biomaterial or as an implant creating a bioactivity.

In these cases, the targets are cosmetology, particularly biostimulant and regenerative skin preparations, medicine, particularly cicatrization as a culture medium favorable to cell multiplication, in implantology as a biomaterial, as such or associated with the surface of an implant to create bioactivity, particularly bioadhesion.

The present invention also concerns, under a fifth aspect, a method for the control of the individual or simultaneous content of chitosan, lipid or calcium in a biological medium, characterized in that it includes the formation in situ of the aforementioned stable ternary complex of one or the other constituents of the ternary complex other than the constituent whose content is to be controlled.

Thus, if it is desired to control the lipid content of a medium, particularly by eliminating the lipid present in the medium, the procedure then is to provide calcium ions and chitosan to form the aforementioned stable ternary complex, which separates by precipitation in case it is the calcium that is to be controlled, particularly by being partially or totally eliminated, and lipid and chitosan are added to form the aforementioned stable ternary complex. It is also possible, for example, to control the simultaneous content of lipid and calcium, particularly in order to eliminate each of these two elements from the medium by adding chitosan alone in sufficient quantity to form the above-mentioned stable ternary complex. All of the possible variants are of course clearly understood by the man of the art and form

an integral part of the present invention.

This process may be applicable to many fields, particularly in the realm of dietetics or in the scope of low-fat diets, in the scope of cosmetology for external thinning preparations for cutaneous application, in the scope of medicine for the treatment of certain lesions, in the scope of water purification used for eliminating lipids from industrial effluents such as those relating to the nutritional field, in the scope of agriculture for the protection of vegetables, seeds, roots, stems, leaves, flowers, fruits, or in the biostimulant area promoting growth factors.

Mention may also be made of the biotechnologies in which the use of the stable ternary complex of the invention is of interest as a medium for animal or vegetable cell culture, or in agriculture in the area of soil improvement.

The ternary complex of the invention has great stability enabling it to withstand a temperature at least equal to 45 °C, which permits the preparation of cosmetic or pharmaceutical compositions requiring stability at a temperature at least equal to 45 °C.

Other purposes, characteristics and advantages of the invention will be clearly apparent to the man of the art from the explanatory description that follows in connection with several examples of preparation given merely for illustration, which should therefore in no way limit the scope of the invention. These examples are given with reference to several figures representing infrared spectra obtained as follows:

- Figure 1 represents the infrared spectrum of fully deacetylated chitosan,
- Figure 2 represents the infrared spectrum of the complex compound obtained by the addition of 5.10^{-4} M chitosan in a solution containing 1.10^{-3} M of undecylenate of sodium and 1.10^{-3} M of calcium chloride,
- Figure 3 represents an infrared spectrum of a chitosan film after 13 days of steeping in a solution of sodium undecylenate 1.10^{-3} containing 1.10^{-3} M of calcium chloride, and
- Figure 4 represents an infrared spectrum of a chitosan film after 9 days of steeping in a suspension of a lecithin liposome (10 mg/ml) containing 1.10^{-3} M of calcium chloride.

Example I: Preparation of ternary complexes of chitosan, calcium ions and fatty acid in solution

I-A Preparation of chitosan solutions

The starting chitosans are produced in France by Aber Technologies. The various lots are purified in the following manner: 4.8 g is dispersed with agitation in 1 liter of distilled (or deionized) water, 1.8 g of acetic acid is added, and dissolution is reached at the end of 1 h. The mixture is filtered under pressure on a Millipore membrane to 0.22 μm. The

perfectly clear solution obtained is then precipitated with dilute ammonia, at pH 8. It is then centrifuged and washed three times in succession, the sediment is disposed in the minimum of water and lyophilized (alternatively, it can be washed with alcohol, filtered and vacuum dried at 50°C). The solid obtained is then characterized for its residual acetylation rate by infrared spectroscopy and its molecular mass by viscosimetry (A. Domard and M. Rinauds, "Preparation and characterization of fully deacetylated chitosan." Int. J. Biol. Macromol., 1987, 5, 49-52.

The purified chitosans can be fully deacetylated by the method described by A. Domard & Coll. (A. Domard and M. Rinauds, "Preparation and characterization of fully deacetylated chitosan." Int. J. Biol. Macromol., 1987, 5, 49-52), and thus polyglucosamines with a degree of acetylation that may be as high as 6,000.

For certain applications, the fully deacetylated chitosans are hydrolyzed by the method proposed by A. Domard & Coll. (A. Domard and N. Cartier, "Glucosamine oligomers: 1. Preparation and characterization" Int. J. Biol. Macromol., 1989, 11, 297-302).

In all cases stock solutions are prepared with a concentration equal to 9×10^{-2} eq/l by dissolving 0.162 g of chitosan in 20 ml of hydrochloric acid 0.05 M.

I-B: Preparation of fatty acid solutions
Example: Undecylenic acid

Undecylenic acid in the form of sodium salt is used without additional purification (purity 98%). A stock solution of sodium undecylenate in 20 ml of water. The concentration is checked by titration and adjusted as needed.

I-C: Preparation of calcium chloride solutions

10^{-3} M stock solutions are prepared by dissolving anhydrous calcium chloride in the necessary amount of water.

I-D: Mixture of products

i. Starting out from a solution of chitosan of 5×10^{-4} eq/l brought to a pH around 5.5 containing 5×10^{-4} eq/l of $CaCl_2$, the 1/1/1 complex is formed preferentially for added sodium undecylenate solutions of less than 1×10^{-4} M. The 2/1/1 complex is mainly formed for undecylenate additions corresponding to concentrations between 1.5×10^{-4} and 2×10^{-4} M. Lastly the 3/1/1 complex is formed preferably beyond concentrations corresponding to 2.5×10^{-4} M.

ii. Starting out from a chitosan solution of 5×10^{-4} eq/l containing 5×10^{-3} mols per

liter of calcium chloride, the 1/1/1 complex is formed preferentially, up to an added undecylenate concentration of 5 x 10^{-4} M.

iii. Starting out from a chitosan solution of 5 x 10^{-4} eq/l containing 5 x 10^{-3} mols per liter of calcium chloride, polynuclear calcium complexes are formed preferentially.

iiii. Starting out from a 1 x 10^{-3} M solution of sodium undecylenate containing 1 x 10^{-3} mol/l of calcium chloride, upon the first addition of chitosan a precipitate is formed corresponding to a ternary association containing less and less calcium per NH_2 function. If conditions are created in which one amine function per two lipids is added and the solution is left standing for two days, the product that has precipitated is filtered and characterized (after washing with distilled water) by microanalysis. It is shown (Fig. 1) that in this case the complexation was followed by an additional adsorption of lipids onto the precipitate and that it is thus possible to remove the lipids from the medium as soon as the ternary complexation process has started, whereas no lipid : chitosan interaction is obtained in the absence of calcium. Another characterization is obtained by IR spectroscopy (Fig. 2). The ternary complex is manifested by the presence of three types of bands: those which can be considered as typical of the lipid (at 2928, 1638, 1411 and 909 cm^{-1}), of chitosan (at 3960, 1148 and 1085 cm^{-1}) and the new ones bound to the formation of the complex (1709 and 1546 cm^{-1}).

iiiii. Another possibility for forming the ternary complex consists in starting with a 5 x 10^{-4} mixture of undecylenate of sodium and 5 x 10^{-4} eq/l of chitosan, to which calcium chloride is added.

Example II: Preparation of ternary complex of chitosan, calcium ion, and fatty acid, starting from solid chitosan

Preparation of a chitosan film

A chitosan film is prepared by dissolving 15 mg of fully deacetylated chitosan in 1.2 ml of acetic acid diluted to 1%. The film is formed by the evaporation in a vacuum oven at 70°C of the solution spread onto a glass plate. It is then detached by immersion in a dilute ammonia and methyl alcohol bath, then vacuum dried at70°C.

Formation of the complex

The films are immersed, with slow stirring, into 20ml of 1 x 10^{-3} M sodium undecylenate solution containing 1 x 10^{-3} M calcium chloride. The ternary complex establishes itself progressively over time. It can be characterized easily by IR spectroscopy (Figure 3) after washing the film at pH7 and drying. As in the preceding example, characteristic bands of chitosan are found (at 3960, 1582, 1148 and 1085 cm^{-1}), of the lipid (at 2930 and 1640 cm^{-1})

and the new ones connected with the formation of the complex (particularly at 1560 cm^{-1}).

In all the cases of Examples I and II the ternary complexes are formed in solutions containing 0.3 M sodium chloride to maintain a constant ionic force facilitating the measurements of free calcium ion concentration, an ionic force which *a priori* plays no part in the stability of the complexes.

Example III: Preparation of ternary complexes of chitosan, calcium ions, and liposomes from solutions of chitosan

III-A Preparation of liposomes of lecithin

A solution containing 100 mg of lecithin in 10 ml of a mixture of chloroform and ethanol (1:1) is evaporated by means of a rotary evaporator, in vacuo, at 40-50°C. The film obtained is dispersed in 10 ml of water. This suspension is subjected to sonication by means of an ultrasonic microprobe (25 W for 20 min). The liquid then appears clear. It is centrifuged for 45 minutes at approximately 10^5 G. The supernatant contains unilaminar vesicles (SUV) whose shape and size are inspected by electronic transmission microscope.

III-B Formation of the ternary complex

If a solution of chitosan (5 x 10^{-2} eq/l) is added to a dilute suspension of lecithin SUV (approximately 7 mg in 40 ml) placed at pH 5.5, a ternary interaction of chitosan, calcium and liposomes is formed. At greater initial pH levels of up to 6.1, the interaction is more effective. The same type of interaction is also obtained with multilaminar liposomes.

Example IV: Preparation of ternary chitosan, calcium ion and liposome complexes from solid chitosan

The liposomes are prepared as in Example III and the chitosan films as in Example II. A chitosan film is steeped in 10 ml of a suspension of lecithin liposomes (10 mg of lecithin/ml) placed at pH 5.5 and at 37°C in the presence of 0.05 M calcium chloride. The liposome solution can be renewed regularly at the end of each cycle, the film is washed with water at pH 7, then vacuum dried. The complex is characterized by IR spectroscopy (Figure 4). The chitosan bands are the same as in Example III, those of the lipid (essentially at 2930 cm^{-1}) and the new ones typical of the complex at 1620 and 1535 cm^{-1}.

In all cases, the mixtures are prepared in the presence of 0.3 M sodium chloride which has no effect on the stability of the complex.

Claims

1. Stable ternary complex of chitosan, lipid and calcium.

2. Ternary complex according to claim 1, characterized in that the lipid is in the form of a monovalent alkaline metal salt, preferably sodium.

3. Ternary complex according to claim 1 or 2, characterized in that the lipid is formed by a saturated or unsaturated fatty acid, particularly a phospholipid such as lecithin.

4. Ternary complex according to any one of claims 1 to 3, characterized in that it is in the form of a polynuclear complex wherein the number of calcium atoms with respect to the number of chitosan or lipid molecules is greater than 1 and less than 10.

5. Ternary complex according to any one of claims1 to 3, characterized in that the molar ratio of lipid : calcium ion : chitosan is 1 : 1 : 1 or 1 : 1 : 1 or 3 : 1 : 1.

6. Method of preparing a stable ternary complex of chitosan, lipid and calcium, characterized in that the chitosan, the said lipid in the form of monovalent alkaline metal salt and calcium ions are brought together.

7. Method according to claim 6, characterized in that the aforesaid bringing together is performed by dissolution or suspension in an aqueous solution, particularly at a pH approximately between 5.5 and 6.5.

8. Method according to claim 6 or 7, characterized in that the aforesaid bringing together comprises the introduction of the chitosan in free amine form, particularly in the solid state, in a solution containing calcium ions and the lipid in the form of monovalent salt.

9. Method according to claim 6 or 7, characterized in that the aforesaid bringing together includes first of all the formation of a liposomal solution of the aforesaid lipids, then calcium ions are added to this solution, then chitosan is added either in the form of a solid in suspension or in a form dissolved in an aqueous solution.

10. Method according to claim 6 or 7, characterized in that the aforesaid bringing together includes first of all the dissolution of chitosan in an aqueous solution whose pH is approximately between 5.5 and 6.5, to which calcium ions are then added, particularly in a concentration near that of the glucosamine residue of the chitosan, and lastly a solution of the aforesaid lipid is added in the form of monovalent salt.

11. Method according to claim 10, characterized in that the amount of lipid added is progressively increased for an identical initial calcium concentration.

12. Method according to any one of claims 6 to 10, characterized in that the initial ratio of calcium ion to the number of NH_2 groups of the chitosan is elevated, being at least equal to 4 and preferably between 4 and 10, thus forming polynuclear complexes obtained with at least 4 calcium ions for one NH_2 group of the chitosan and 1 mol of lipid.

13. Method for stabilizing an aqueous dispersion of liposomes, characterized in that a stable ternary complex is formed the addition of chitosan and calcium ions to the said suspension of liposomes so as to form a stable ternary complex of chitosan and calcium with the lipids constituting the liposomes.

14. Aqueous dispersion of stabilized liposomes, characterized in that the lipid is in the form of a stable ternary complex of lipids, chitosan and calcium.

Captions of the drawings:

Fig. 1 Infrared spectrum of fully deacetylated chitosan

Fig. 2 Infrared spectrum of the complex obtained by addition of 5.10^{-4} M chitosan to a solution containing 1.10^{-3} M undecylenate of Na and 1.10^{-3} M $CaCl_2$.

Fig. 3 Infrared spectrum of a chitosan film after 13 days of steeping in a solution of undecylenate of Na 1.10^{-3} M containing 1.10^{-3} M $CaCl_2$

Fig. 4 Infrared spectrum of a chitosan film after 9 days of steeping in a suspension of lecithin liposomes (10 mg/ml) containing 1.10^{-3} M $CaCl_2$

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SEARCH REPORT

prepared on the basis of the last claims filed before search began FR 9011746
FA 448364

DOCUMENTS CONSIDERED PERTINENT

Category	Listing of document showing, if necessary. the pertinent parts	Relevant claims of the application in question
A	US-A-4,223,023 (FURDA) Col. 1, lines 39-68; columns 3, 4, example 3	1,3,6,8
A	EP-A-0 183 536 (IHARA CHEMICAL IND. CO.) Page 37, line 5 - page 38, line 25	1,3,6,13,14
A	PATENT ABSTRACTS OF JAPAN, vol. 9, No. 74 (C-273)[1997], 3 April 1985, page 161; & JP-A-59 210 013 (AJINOMOTO) 28-11-1984 Abstract	1,3,6,13,14

TECHNICAL DOMAINS SEARCHED
(International class 5)

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MAZET, J.-F.